Supporting Information

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SI Materials and Methods

Mice Experiments. All animal studies were approved by the Animal Care and Use Committee of the Kyoto Prefectural University of Medicine. p53-deficient mice (C57BL/6J background) and Parkin-deficient mice (1) (C57BL/6J background) were obtained from the Jackson Laboratory. Double heterozygous pairing produced $p53^{+/+}$ $Parkin^{+/+}$, $p53^{-/-}Parkin^{+/+}$, $p53^{+/+}Parkin^{-/-}$, and $p53^{-/-}Parkin^{-/-}$ mice. Eight-week-old mice underwent bone marrow (BM) transplantation and 4 wk later were intraperitoneally injected with five consecutive daily injections of 40 mg/kg of streptozotocin (STZ). C57BLKS/J *db/db* mice were obtained from Japan SLC, Inc. pifithrin-α (PFT-α) was intraperitoneally administered at a dose of 1.1 mg/kg three times weekly for 4 wk. Animals were maintained in a specific pathogen-free animal facility on a 12-h light–dark cycle at an ambient temperature of 21 °C. They were given free access to water and food. Age- and sex-matched mice were used for all animal experiments.

Bone Marrow Transplantation. Total BM hematopoietic progenitor donor cells were harvested from WT–GFP and $p53^{-/-}$ mice and transplanted via a tail-vein injection into lethally irradiated (9 Gy) recipient mice. The minimum cell dose was 2.0×10^6 cells per mouse. Transplanted mice were housed for 4 wk before the STZ injection.

Metabolic Studies. For insulin tolerance tests, mice were fasted for 3 h, after which blood glucose concentrations were assessed before an i.p. injection of 1 and 2 U/kg of insulin into C57BL/6J and *db/db*, respectively, and then 15, 30, 60, and 120 min after the injection. At each time point, a 5-μL blood sample was collected via a tail nick, and glucose was assessed using an automatic blood glucose meter. For oral glucose tolerance tests, mice were fasted for 16 h, after which blood insulin and glucose concentrations were assayed before the oral administration of 1 and 0.5 g/kg glucose to C57BL/6J and *db/db* mice, respectively. Glucose levels were then assessed 15, 30, 60 and 120 min after the glucose administration, and insulin levels were assessed 15 and 30 min after the glucose administration using an ELISA kit (Morinaga).

Palmitate Solution. Stock solutions were prepared as follows: palmitic acid (Sigma) was dissolved in 75% ethanol at 70 °C at a final concentration of 300 mM. Aliquots of stock solutions were complexed with fatty-acid–free BSA (10% solution in 150 mM NaCl; Sigma) by stirring for 1 h at 37 °C. The final molar ratio of fatty acid:BSA was 5:1. The final ethanol concentration of stock solution was 1.5% (vol:vol). All control conditions included a solution of vehicle (ethanol:H_2O) mixed with fatty-acid–free BSA in NaCl solution at the same concentration as the palmitate solution.

Plasmids and RNA Interference. Mouse p53, Parkin, advanced-glycation end products (RAGE), Toll-like receptor 4 (TLR4), p47phox, Atg5, and PINK1-specific siRNA duplexes were purchased from Invitrogen. siRNAs were transiently transfected using Lipofectamine RNAiMAX (Invitrogen) according to the product protocol.

Generation of Stable MIN6 β-Cell Lines. The Parkin transcript was amplified from mouse heart total RNA using Parkin-specific primers. Parkin cDNA was cloned into a pcDNA3.1 vector. Empty vectors and vectors carrying Parkin were transfected to MIN6 β-cells using Lipofectamine 2000 (Invitrogen) according to the product protocol. After selection with 500 $\mu g \cdot ml^{-1}$ of G418, resistant colonies were isolated.

Nuclear and Cytosolic Fraction Preparation. Isolated islets or MIN6 β-cells were homogenized with lysis buffer [10 mM Hepes–KOH (pH 7.9), 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM DTT, 1% Nonidet P-40, supplemented with mammalian protease inhibitor mixture (Sigma)]. Homogenates were centrifuged at $800 \times g$ for 10 min. The supernatants were further centrifuged at $12{,}000 \times g$ for 10 min, and the resultant supernatant was used as the cytosolic fraction. The nuclear pellet was washed with lysis buffer and then sonicated. After centrifugation at $12{,}000 \times g$ for 10 min, the supernatant was used as a crude nuclear fraction.

Adenovirus. Adenovirus expressing GFP-LC3 was a kind gift from T. Yoshimori (Osaka University, Osaka). Cells were infected with adenovirus that was diluted in the culture medium at a multiplicity of infection of 50 and incubated at 37 °C for 1 h.

Immunoblot and Immunoprecipitation. Antibodies used for immunoblotting included gout anti-p53 (1:500, C-19, Santa Cruz); mouse anti-p53 [1:1,000, #2524, Cell Signaling Technology (CST)]; rabbit anti-Parkin (1:1,000, ab15954, Abcam); mouse anti-Parkin (1:1,000, #4211, CST); mouse anti-GAPDH (1:5,000, MAB374, Millipore); rabbit anti-histone H3 (1:5,000, ab1791, Abcam); rabbit anti-PERK (1:1,000, #3192S, CST); rabbit anti-pPERK (1:500, #9101S, CST); mouse anti-GSK3β (1:500, 610210, BD); and rabbit anti-pGSK3β (1:1,000, #9336S, CST). HRP-conjugated secondary antibodies were obtained from Amersham Pharmacia and Millipore. PVDF membrane (BioRad) was used for blotting and signals were revealed by ECL prime (GE Healthcare). For immunoprecipitation, MIN6 β-cells were treated in lysis buffer (50 mM Tris·HCl, 150 mM NaCl, 1% Nonidet P-40, 1 mM DTT, 1 mM EGTA, 1 mM Na_3VO_4, and 50 mM NaF, supplemented with mammalian protease inhibitor mixture) (Sigma). Cell lysates (200 μg protein) were subjected to immunoprecipitation with antibodies coupled to magnetic beads (Magnosphere MS300/Carboxyl, JSR). After the beads were washed, samples were boiled with sample buffer and subjected to Western blotting.

RNA Analysis. Total RNA was extracted from cultured MIN6 β-cells using TRIzol reagent (Invitrogen) followed by RNeasy clean-up (Qiagen) and then reverse-transcribed using a Prime Script RT reagent kit with gDNA Eraser (Takara). Quantitative real-time reverse transcription-PCR was performed using SYBR Premix Ex Taq II (Tli RNaseH Plus) (Takara) with the Thermal Cycler Dice Real Time System (Takara) according to the manufacturer's suggestions. The expression levels of target genes were normalized by the expression levels of housekeeping gene EIF3F (TIF).

Islet Morphometry, Immunohistochemistry, and Immunofluorescent Staining. Isolated pancreata were fixed in 4% paraformaldehyde at 4 °C overnight. Tissue was then routinely processed for paraffin embedding, and 2-μm sections were cut and mounted on glass slides. These sections were immunostained using guinea pig anti-porcine insulin antibody (1:800, A05642, Dako) or rabbit anti-proliferating cell nuclear antigen (PCNA) antibody (1:40, sc-7907, Santa Cruz) and counterstained with hematoxylin. TUNEL was performed using immunohistochemistry. Images of pancreatic tissue were captured on a Keyence BZ-9000 microscope. The area of β-cells relative to the total area of pancreatic tissue was

calculated with BZ-Analyzer software (Keyence). Cultured MIN6 β-cells were fixed with 4% paraformaldehyde, permeabilized with methanol/acetone, and stained with the following antibodies: rabbit anti-TOM20 (1:200, sc-11415, Santa Cruz) and mouse anti-p53 (1:100, #2524, CST). They were then mounted using VectaShield with DAPI mounting medium (H-1200; Vector Laboratories) and observed under a fluorescence confocal microscope (LSM510 META, Carl Zeiss).

Electron Microscopy. The pancreas was fixed in 2% glutaraldehyde with 0.1 mM phosphate buffer (pH 7.2) for 24 h at 4 °C, postfixed in 2% osmium tetroxide with 0.1 mM phosphate buffer (pH 7.2) for 120 min at 4 °C, and then serially dehydrated in ethanol and embedded in epoxy resin. Sections were cut on an LKB ultramicrotome, and consecutive ultrathin sections were mounted on copper grids. Ultrathin sections were stained with 3% uranyl acetate and 0.2% lead citrate. Examinations were conducted with an electron microscope (JEM-1200EX, JOEL). Analysis of damaged mitochondria and autophagosomes was made from 20 cells. Damaged mitochondria were defined by the swelling associated with an increased number of disarrayed or disappeared cristae and by the reduced electron density of the matrix. Autophagosomes were identified by the characteristic structure of a double or multilamellar smooth membrane completely surrounding compressed mitochondria or membrane-bound electron-dense material.

Mitochondrial Isolation. MIN6 β-cells were homogenized in ice-cold MSE buffer [220 mM mannitol, 70 mM sucrose, 2 mM EGTA, 5 mM Mops (pH 7.4), 2 mM taurine, and 0.2% BSA]. The homogenate was centrifuged at 500 × *g* twice for 5 min, saving the supernatant. Pellet mitochondria was obtained from the centrifugation of the supernatant at 3,000 × *g* twice and was rinsed with MSE buffer. The final pellet was resuspended in 50 μL of incubation medium [220 mM mannitol, 70 mM sucrose, 1 mM EGTA, 5 mM Mops (pH 7.4), 2 mM taurine, 10 mM $MgCl_2$, 5 mM KH_2PO_4, and 0.2% BSA]. Mitochondria were incubated for 15 min on wet ice, and protein concentrations were determined with BSA as a standard by the Bradford assay. All work was performed on wet ice.

Oxygen Consumption Measurements. Oxygen consumption of islets and isolated mitochondria was measured as previously described using XF24 (Seahorse Bioscience) (2, 3). To measure oxygen consumption of isolated islets, ~30 islets were suspended in modified XF assay medium (2.8 mM glucose, 0.8 mM Mg2+, 1.8 mM $CaCl_2$, 143 mM NaCl, 4.4 mM KCl, 0.91 mM KH_2PO_4, FBS 1%, phenol red 15 $\mu g \cdot ml^{-1}$) and then transferred into the depressed chamber. To stimulate islets, 20 mM glucose was used. To block F1Fo-ATPase, 5 μM of oligomycin was used. After the XF24 calibration was complete, the islet plate was placed into the XF24, and then the program was started. Basal respiration rates were used for normalization. To measure oxygen consumption in isolated mitochondria, 20 μg mitochondria was suspended in 50 μL of mitochondrial assay solution [MAS: sucrose 70 mM, mannitol 220 mM, KH_2PO_4 5 mM, $MgCl_2$ 5 mM, Hepes 2 mM, EGTA 1 mM, BSA fatty-acid–free 0.2% (pH 7.4) adjusted with KOH 1 M] and loaded in the V7 plate. After loading, centrifugation of the V7 plate for 20 min at 2,000 × *g* (4 °C) was performed to attach mitochondria at the bottom of the plate. To analyze sequential electron flow through different complexes of the electron transport chain, 400 μL of MAS + 0.5 mM NADH and 4 μM carbonylcyanide-p-trifluoromethoxyphenylhydrazone were added on top. The loaded V7 plate was incubated for 5 min at 37 °C (no CO_2 incubator) before loading it into the XF24. Inhibitors and substrates were injected as follows: port A, 50 μL of 20 μM rotenone (2 μM final); port B, 55 μL of 100 mM succinate (10 mM final); port C, 60 μL of 40 μM antimycin A (4 μM final); port D, 65 μL of 100 mM ascorbate plus 1 mM N,N,N′,N′-tetramethyl-p-phenylenediamine (10 mM and 100 μM final, respectively).

Static Insulin Secretion from Isolated Islets. Pancreatic islets were isolated as previously described by collagenase injection into the bile duct (4). Briefly, islets were isolated using collagenase XI (Sigma) (5), digested in Hanks' buffer, followed by the separation of islets from exocrine tissue in a Histopaque 1077 (Sigma) gradient. Islets of similar sizes were handpicked under a stereomicroscope into groups of 5–10 islets in triplicate. Islets were preincubated for 60 min at 37 °C in Krebs–Ringer bicarbonate Hepes buffer containing 129.4 mM NaCl, 3.7 mM KCl, 2.7 mM $CaCl_2$, 1.3 mM KH_2PO_4, 1.3 mM $MgSO_4$, 24.8 mM $NaHCO_3$ (equilibrated with 5% CO_2, 95% O2, pH 7.4), 0.2% (vol/vol) BSA (fraction V), and 2.8 mM glucose. After preincubation, five islets were incubated with 200 μL of the same buffer for 30 min. After the buffer was removed to measure insulin, islets were incubated in the presence of 22.2 mM of glucose for another 30 min. At the end of this period, the buffer was collected. These buffers were stored at −80 °C until the insulin assay with the Morinaga mouse insulin assay kit (Morinaga Institute of Biological Science).

Insulin Secretion Assay in MIN6 β-Cells. Stable MIN6 β-cells (2.0×10^5 cells per 24-well plate) carrying Parkin or empty vector were cultured in DMEM supplemented with 10% FBS containing 30 mM glucose/0.3 mM palmitate or 10 mM glucose/vehicle (BSA) for 1 wk, after which glucose-stimulated insulin secretion was analyzed as described for isolated islets.

ATP Content Measurement. MIN6 β-cells (2.0×10^5 cells per 24-well plate) or isolated pancreatic islets were preincubated at 37 °C for 60 min in Krebs–Ringer bicarbonate Hepes buffer with 2.8 mM glucose and then were incubated at 2.8 or 22.2 mM glucose for another 30 min. ATP content was measured using an ATP bioluminescent assay kit (TOYO Ink).

Glucokinase and Hexokinase Assay. MIN6 β-cells (2.0×10^5 cells per 24-well plate) were preincubated in Krebs–Ringer bicarbonate Hepes buffer with 2.8 mM glucose for 2 h and then homogenized with 250 μL of extract solution consisting of 20 mM KH_2PO_4, 100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, and 1 mM DTT (pH 7 by KOH), and the supernatant was obtained from the homogenate by centrifugation at 10,000 × *g* for 15 min. The glucose phosphorylation rate was estimated as the increase in NADH through the following reaction: glucose-6-phosphate + NAD 6-phosphoglucono-δ-lactone + NADH by NAD-dependent G6PDH. The enzyme reaction was performed using 12.5 μL of extract in 80 μL of a reaction solution consisting of 25 mM Hepes, 25 mM KCl, 2.5 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, 1 mM thio-NAD, 100 U/mL G6PDH (pH 7.4 by KOH) supplemented with two concentrations (50 mM for glucokinase and 0.5 mM for hexokinase) of glucose at 37 °C for 1 h. The reaction was stopped by adding 145 μL of stop solution consisting of 300 mM Na_2HPO_4, 0.46 mM SDS (pH 8.0). NADH concentration was measured by the fluorescence spectrophotometer (Tecan, Infinite F200) at excitation and emission of 360 and 465 nm, respectively. Blanks in the absence of ATP were incubated in a parallel experiment. Glucokinase activity was calculated, subtracting hexokinase activity measured at 0.5 mM glucose from the activity measured at 50 mM glucose.

NADPH Oxidase Activity. NADPH oxidase activity was evaluated using lucigenin chemiluminescence as previously described (6). Briefly, MIN6 β-cells (2.0×10^5 cells per 24-well plate) were homogenized in Krebs–Hepes buffer and centrifuged at 10,000 × *g* for 15 min. The supernatants were transferred

to a 96-well plate containing 5 μM lucigenin (Sigma) in the same buffer and incubated in the dark for 10 min at 37 °C. The chemiluminescence was measured over the subsequent 1 min in response to the addition of 100 μM NADPH using the luminescence channel of a Tecan Infinite F200 plate reader. Data are expressed as relative light units per minute per milligram protein.

Quantification of Mitophagy. MIN6 β-cells (3.0×10^4 cells per 96-well plate) were transfected with pMT-mKeima-Red (Amalgaam) using Lipofectamine 2000 (Invitrogen). After a subsequent 1-wk incubation in control or high-glucose/palmitate-containing medium, the 96-well plates were read at 430 and 590 nm (excitation) using the fluorescence spectrophotometer (Tecan, Infinite F200). The 590/430-nm ratios were calculated from each well and were used as an index for mitophagy.

Flow Cytometry. Mitochondrial reactive oxygen species (ROS) content in MIN6 β-cells was evaluated using CM-H_2DCFDA (Molecular Probes). Samples were analyzed by FACSCaliber (Becton Dickinson) using FlowJo Software (Treestar).

Statistical Analysis. Data are shown as the mean ± SD. Statistical comparisons were made using the two-tailed unpaired Student *t* test. A probability value of <0.05 was considered to indicate statistical significance. Statistical analysis was performed using Excel software.

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2. Rogers GW, et al. (2011) High throughput microplate respiratory measurements using minimal quantities of isolated mitochondria. *PLoS ONE* 6(7):e21746.
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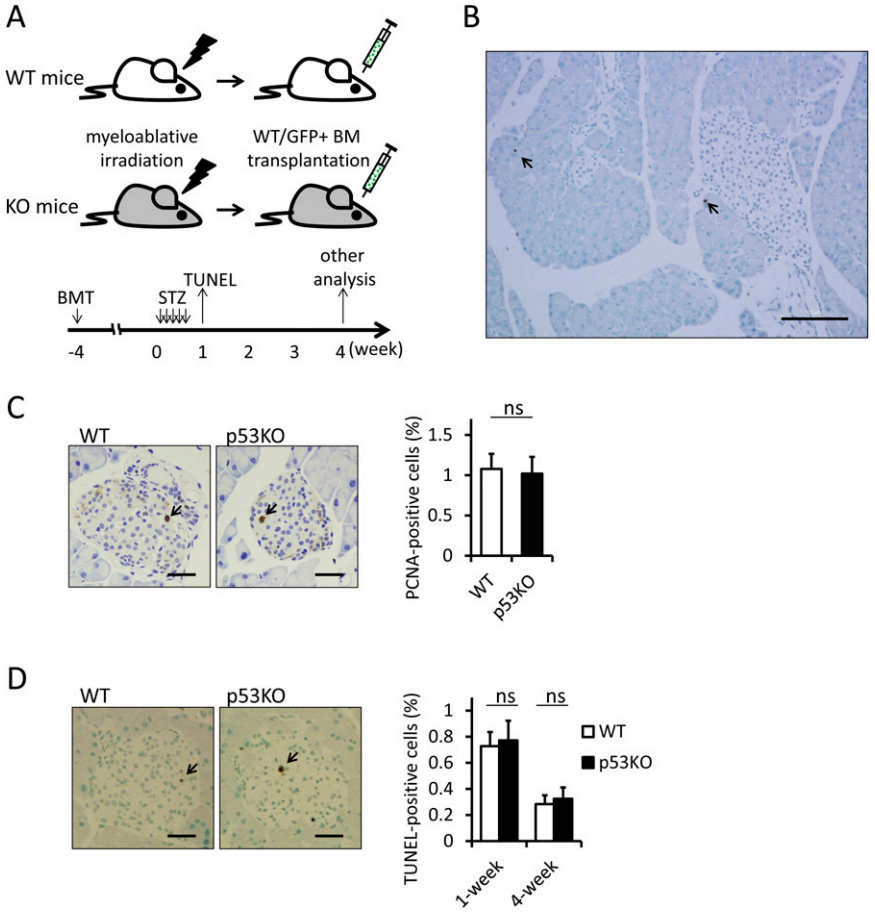

Fig. S1. Protocol for STZ-induced type 1 diabetic model and histological analysis of islets in STZ-treated mice (related to Figs. 1 and 2). (*A*) Eight-week-old mice underwent BM transplantation from WT-GFP mice and were intraperitoneally injected with five consecutive daily injections of 40 mg/kg of STZ 4 wk later. (*B*) TUNEL-positive apoptotic cells were determined 1 d after irradiation. A few TUNEL-positive cells were observed in the extra-islet area. Arrows indicate TUNEL-positive cells. (Scale bars, 100 μm.) (*C*) Histological analysis of PCNA immunostaining of islets 4 wk after STZ treatment. (*Right*) The percentage of PCNA-positive cells per total islet cells. (Scale bars, 40 μm.) (*D*) Representative images of TUNEL staining 1 wk after STZ treatment. The percentage of TUNEL-positive apoptotic cells per total islets cells is determined both 1 and 4 wk after STZ treatment. (Scale bars, 40 μm.) ($n = 4$). Arrows indicate PCNA- or TUNEL-positive cells. Data are shown as the means ± SD.

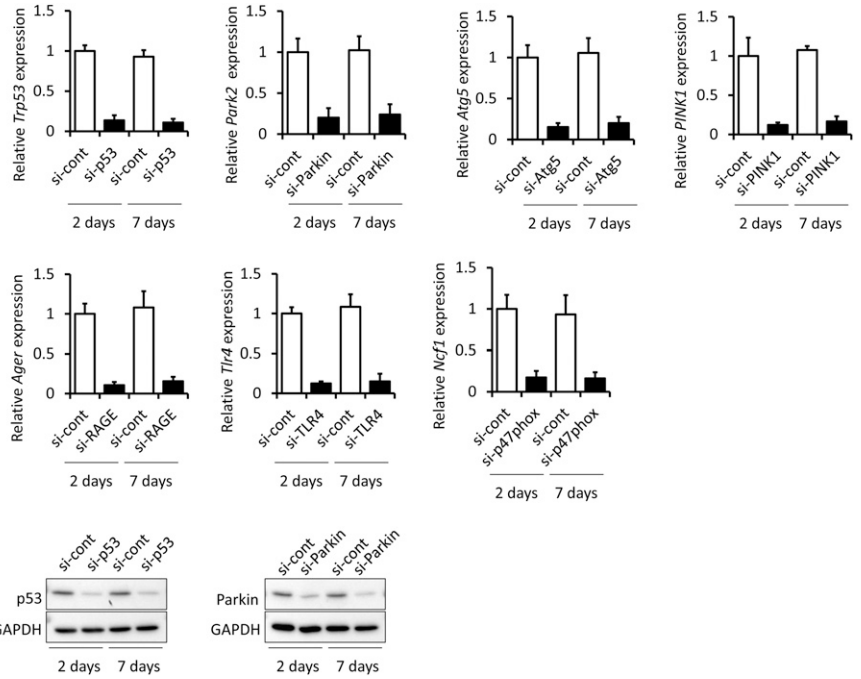

Fig. S2. The gene-silencing effect of siRNAs used in this study. The extent of knockdown at the mRNA levels was confirmed on both 2 and 7 d after transfection because MIN6 cells were incubated in high-glucose and palmitate-containing buffer for 1 wk. The efficacy of knockdown of p53 and Parkin was also assessed at the protein levels. RT-PCR data are assessed as the ratio of mRNA expression of each molecule to mRNA expression of TIF and expressed as the fold change relative to the value of control cells. Results are shown from three independent experiments. Data are shown as the means ± SD.

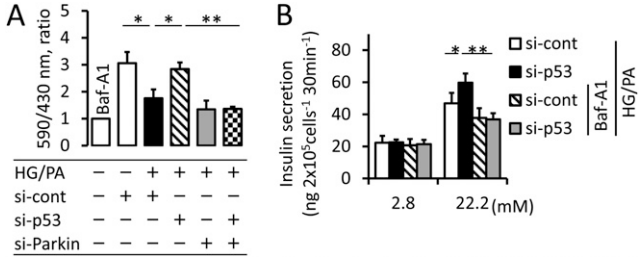

Fig. S3. Quantification of mitophagy and insulin secretion analysis (related to Fig. 3). pMT-mKeima-Red is a mitochondria-targeted, coral-derived, acid-stable fluorescent protein that exhibits a bimodal excitation maximum at 430 and 590 nm in a neutral or acidic pH, respectively. (*A*) Excitation ratio (590/430 nm) of pMT-mKeima-Red–overexpressing MIN6 β-cells incubated with high glucose (HG) and palmitate (PA). A high 590/430-nm ratio is indicative of increased mitophagy. Bafilomycin-A1 (Baf-A1)-treated cells were used as a negative control for the induction of mitophagy. The experiments were performed in eight replicates ($n = 3$). (*B*) Glucose-stimulated insulin secretion was measured by ELISA in the context of mitophagy deficiency induced by the Baf-A1 treatment. The experiments were performed in duplicate ($n = 4$). Data are shown as the means ± SD. *$P < 0.05$; **$P < 0.01$.

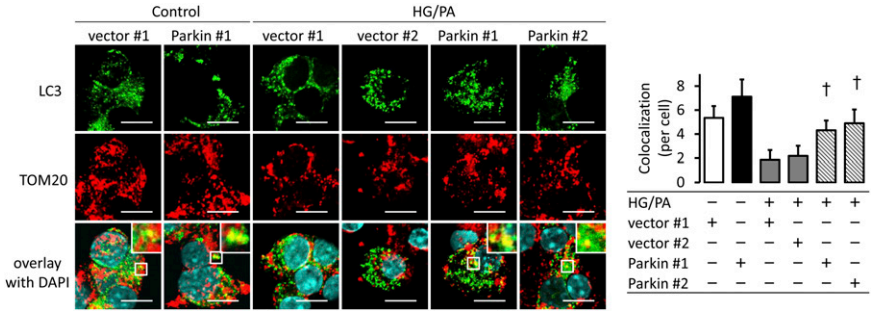

Fig. S4. Mitophagy in Parkin-overexpressing stable cell lines (related to Fig. 4). Representative images of GFP-LC3–expressing MIN6 β-cells exposed to HG and PA. We generated MIN6 β-cells stably overexpressing Parkin. Cells incubated with HG and PA were infected with GFP-LC3 adenovirus. Baf-A1 treatment was performed to underscore mitophagy for 6 h before the immunostaining of mitochondria with anti-TOM20 (red) (original magnification, ×1,000). (Scale bars, 10 μm.) (*Right*) Colocalization between LC3 and TOM20. A minimum of 50 GFP-positive cells were scored in three independent experiments. Data are shown as the means ± SD. $^{\dagger}P < 0.05$ versus stable cell lines carrying control vectors.

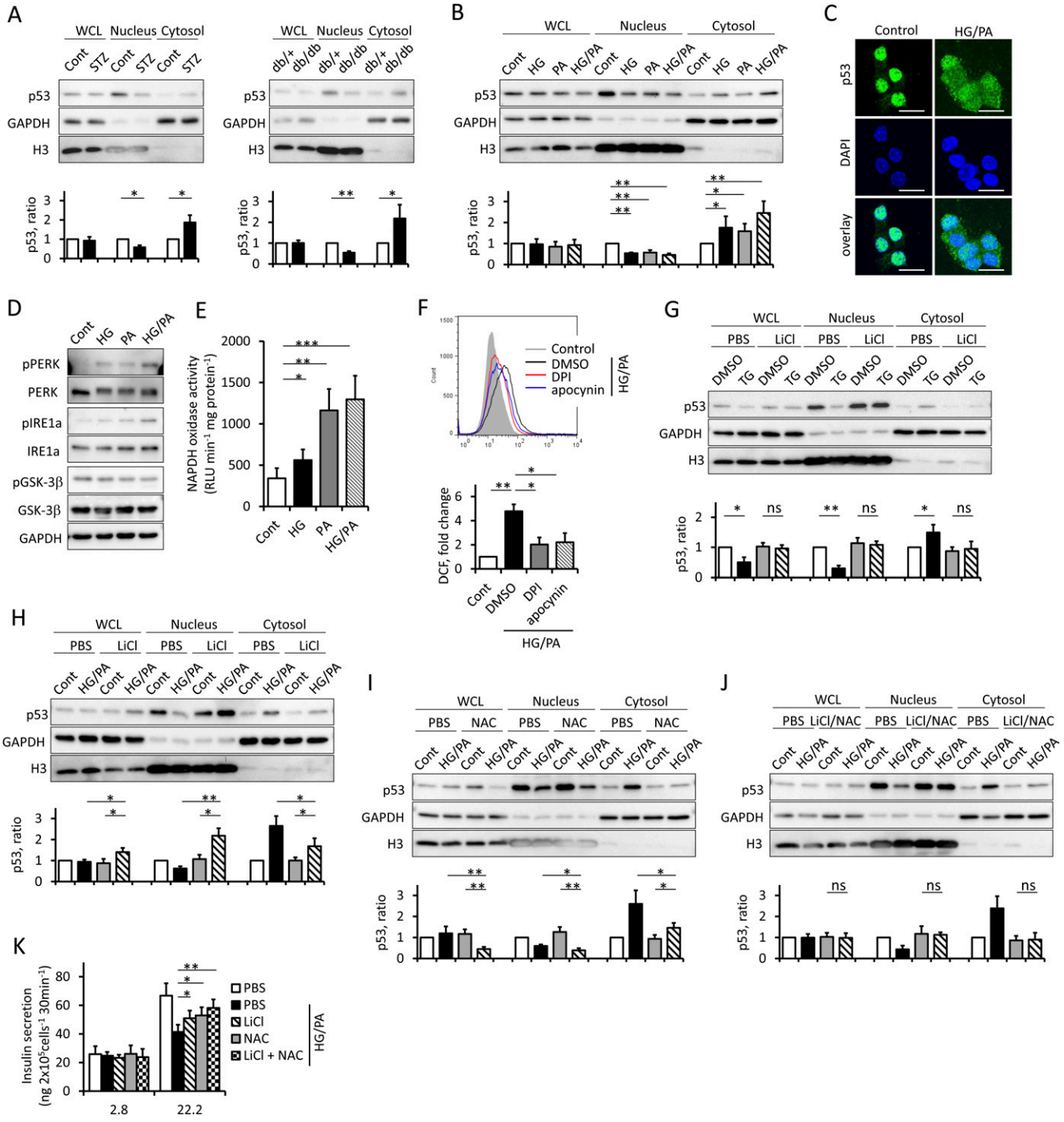

Fig. S5. Cytosolic p53 is up-regulated under diabetic conditions through endoplasmic reticulum (ER) stress and oxidative stress. (*A*) Subcellular fractionation of islets from STZ-treated mice and *db/db* mice. Whole-cell lysates (WCL), nuclear lysates (Nucleus), or cytosolic lysates (Cytosol) were subjected to immunoblotting. (*B*) Subcellular fractionation of MIN6 β-cells. Cells were incubated with 30 mM HG and/or 0.3 mM PA for 1 wk. (*C*) Subcellular localization of p53 was determined by immunostaining with anti-p53 (green) (original magnification, ×1,000) (Scale bars, 15 μm.) (*D*) MIN6 β-cells were incubated with HG and/or PA for 1 wk and were then subjected to immunoblotting. Representative immunoblots from three independent experiments are shown. (*E*) NADPH oxidase activity was evaluated using the lucigenin chemiluminescence assay in MIN6 β-cells incubated with HG and/or PA for 1 wk in duplicate ($n = 4$). (*F*) ROS production was examined by flow cytometry for CM-H_2DCFDA. MIN6 β-cells exposed to HG and PA were treated with NADPH oxidase inhibitors, 10 μM diphenyleneiodonium chloride (DPI), 1 mM apocynin, or the carrier DMSO in duplicate ($n = 3$). (*G*) Subcellular fractionation of MIN6 β-cells that were treated with 1 μM of thapsigargin (TG) and/or 20 mM lithium chloride (LiCl), an inhibitor of GSK-3. (*H*) Subcellular fractionation of MIN6 β-cells that were treated with 20 mM LiCl. (*I*) Subcellular fractionation of HG- and PA-incubated MIN6 β-cells that were treated with 20 mM *N*-acetylcysteine (NAC). (*J*) Subcellular fractionation of MIN6 β-cells that were treated with 20 mM LiCl and 20 mM NAC. (*K*) Glucose-stimulated insulin secretion was measured by ELISA in the context of inhibiting p53 redistribution by LiCl and/or NAC treatment. The experiments were performed in duplicate ($n = 4$). (*A*, *B*, and *G*–*H*) Representative immunoblots are shown, and p53 levels are quantified as the ratio relative to GAPDH or H3 levels from three independent experiments. Data are shown as the means ± SD. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

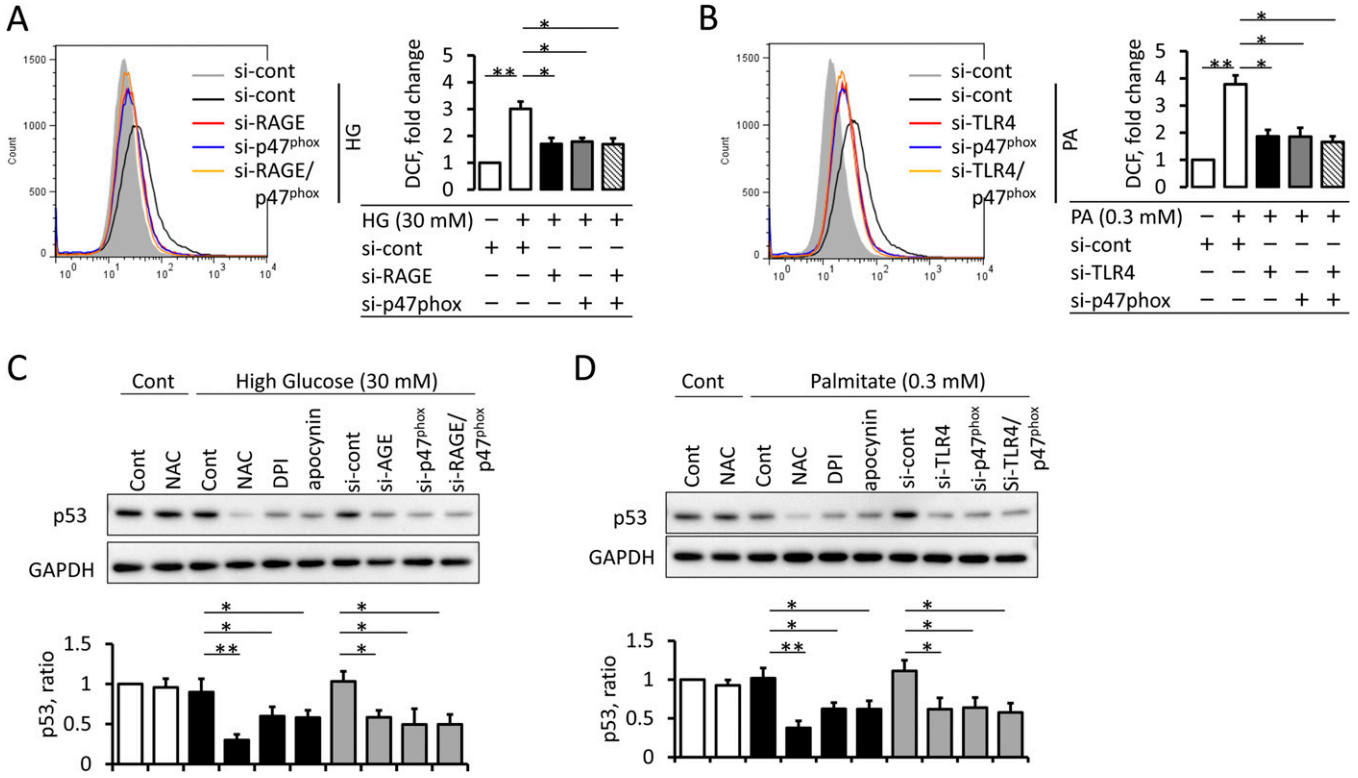

Fig. S6. Both glucotoxicity and lipotoxicity activate NADPH oxidase. (*A* and *B*) ROS production was examined by flow cytometry for CM-H_2DCFDA. MIN6 β-cells exposed to HG were transfected with siRNA targeting RAGE and/or p47phox (*A*). MIN6 β-cells exposed to PA were transfected with siRNA targeting TLR4 and/or p47phox (*B*). The experiments were performed in duplicate ($n = 3$). (*C*) p53 expression was determined in HG-exposed MIN6 β-cells that were treated with 20 mM NAC, 10 μM DPI, or 1 mM apocynin or were transfected with siRNA targeting RAGE and/or p47phox. (*D*) p53 expression was determined in PA-exposed MIN6 β-cells that were treated with 20 mM NAC, 10 μM DPI, or 1 mM apocynin or were transfected with siRNA targeting TLR4 and/or p47phox. (*C* and *D*) Representative immunoblots are shown, and p53 levels are quantified as the ratio relative to GAPDH or H3 levels from three independent experiments. Data are shown as the means ± SD. $*P < 0.05$; $**P < 0.01$.

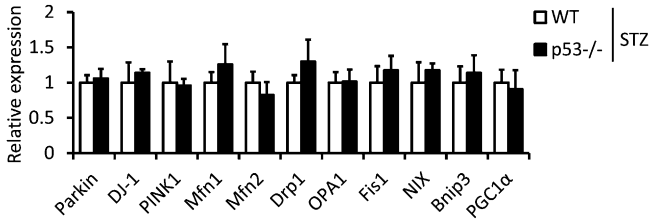

Fig. S7. Mitochondrial quality control factors in STZ-treated WT and $p53^{-/-}$ mice. The mRNA expressions of molecules associated with mitochondrial quality control were analyzed in islets of STZ-treated mice. Data are assessed as the ratio of mRNA expression of each molecule to mRNA expression of TIF and expressed as the fold change ($n = 4$). Data are shown as the means ± SD.

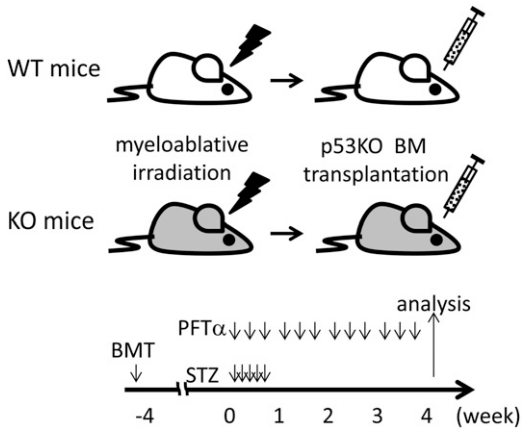

Fig. S8. Protocol for PFT-α experiments in STZ-induced type 1 diabetic model (related to Fig. 5). Eight-week-old mice underwent BM transplantation from *p53*$^{-/-}$ mice and were intraperitoneally injected with five consecutive daily injections of 40 mg·kg^{-1} of STZ at 4-wk intervals. PFT-α and the carrier DMSO were injected three times per week for 4 wk starting on the first day of STZ treatment.

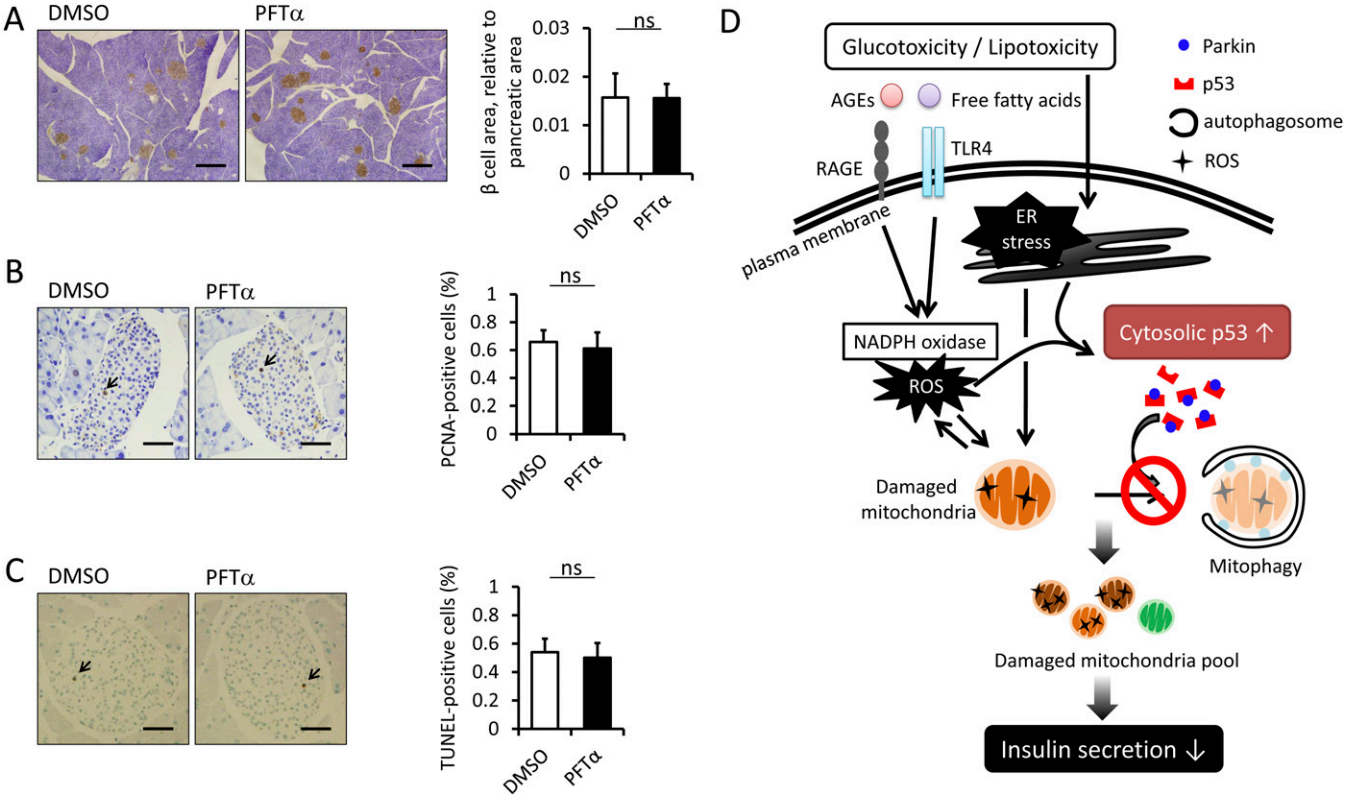

Fig. S9. β-Cell mass, cell proliferation, and apoptosis in *db/db* mice (related to Fig. 6). (*A* and *B*) Histological analysis of insulin (*A*) and PCNA (*B*) immunostaining of islets in *db/db* mice were performed after 4 wk of treatment with PFT-α. (*Right*) The islet β-cell area relative to pancreatic area and percentage of PCNA-positive cells per total islet cells. (Scale bars: *A*, 500 μm; *B*, 60 μm.) $n = 3$. (*C*) TUNEL-positive apoptotic cells were determined after 4 wk of treatment with PFT-α. (*Right*) The percentage of TUNEL-positive cells per total islet cells. (Scale bars, 60 μm.) $n = 3$. (*D*) A model for mitophagy deficiency in diabetes. Glucolipotoxicity induces ROS production via RAGE and TLR4 as well as ER stress. Oxidative stress and ER stress combine to induce the cytosolic accumulation of tumor suppressor p53. Cytosolic p53 disturbs Parkin translocation to damaged mitochondria, leading to mitophagy deficiency and subsequent mitochondrial compromise. As a result, the mitochondria-dependent insulin secretion against blood glucose elevations is impaired in diabetes.